The invention will now be illustrated but not limited by reference to the following Figures and Examples wherein:

Figure 1 shows the basic features of a convenient primer design, the template binding region is indicated by the shaded arrow, the tail region comprises a blocking group indicated by H, also shown are a quencher and fluorophore, the target binding region is in the region indicated by the solid line between the quencher and fluorophore.

Figure 2 shows quenching achieved by random coiling of the tail bringing the fluorophore and quencher pair into close proximity.

Figure 3 shows hybridisation of the target binding region to a complementary sequence in the primer extension product corresponding to the target region.

Figure 4 (a) shows the inclusion of an intercalating fluorophore (IF) in the tail of the primer and primer extension on a sample template, (b) shows intercalation after hybridisation of the target binding region to a complementary sequence in the primer extension product corresponding to the target region.

Figure 5 (a) shows the use of dyes (R & F) incorporated into the primer and which form an energy tranfer pair, (b) shows their relative position upon hybridisation.

Figure 6 (a) shows the use of a primer having a single fluorophore (F) attached at the 5' terminus, a blocking group (H) is shown, the target binding region is indicated by the arrow to the right, (b) shows the random coiling and quenching of the fluorophore in solution and (c) shows hybridisation of the target binding region after primer extension.

Figure 7 (a) shows the bimolecular embodiment of the invention, the fluorophore and quencher are provided on separate species, in (b) the primer is extended on a sample template, and in (c) separation of the fluorophore and quencher upon hybridisation of the target binding region are shown.

Figure 8 (a) shows the capture probe embodiment of the invention, in (b) amplicons are captured on a solid phase and probed using the same non-amplifiable tail, in (c) the primer comprises a branched structure of the tail and capture sequences.

Figure 9 shows the stem embodiment of the invention, (a) at high temperatures, the stem duplex is disrupted and the fluorophore is unquenched, ie. "on"; (b) at lower temperatures, however, the stem duplex forms and the fluorescence is substantially off.

Figure 10 shows the primer as used in an amplification cycle. (a) after initial denaturation, annealing and extension, the *Scorpions* amplicon comprises a region complementary to the loop region at its 5'-end; (b) upon a second round of denaturation and annealing, the tail hybridises (c) to the newly synthesised region with great efficiency (a unimolecular interaction) and fluorescence remains unquenched (Figure 10c). Unextended primers, however, will continue to form their quenched conformation.

Figure 11 shows use of the primer as (a) a common primer in a two tube ARMS test and (b) as allele specific primers "a" and "b" in a single tube ARMS test.

Figure 12 shows the use of the primer where hybridisation of the target binding region occurs in an allele specific manner, in (a) primer extension gives a product corresponding to allele "a" or "b", in (b) hybridisation is allele specific or mismatched in (c) and (d) probes for each variant are provided on each of the two amplimers, thereby probing different strands of the reaction, and in (e) different primers may be used in the same mix for allele discrimination and as control primers for amplicon detection.

Figure 13 shows real time detection of amplification, fluorescent signal is generated upon hybridisation of a matched target binding region in contrast to a mismatched target.

Figure 14 shows allele discrimination, fluorescent signal is generated upon hybridisation of a matched target binding region in contrast to a no-template control and a mismatched target.

Figure 15 shows primer titration, fluorescent signal is generated upon hybridisation of a matched target binding region in contrast to a mismatched target. The following proportions of Scorpion primer were used: (a) 100%, (b) 80%, (c) 50%, (d) 20% and (e) 10%.

Figure 16 shows heteroplasmy analysis, varying admixtures of C homozygote and A homozygote were used as shown and readings taken after 40 cycles of PCR.

Figure 17 shows a comparison between this invention and a bimolecular equivalent. In (a) mismatched targets show no appreciable amplification, in (b) and (c) a substantial allele specific signal is produced only by the matched Scorpions primers. In Figure 17 results obtained using Scorpions primers are shown as triangles and crosses.

Figure 18 shows use of the no quencher embodiment of the invention, fluorescent signal is generated upon hybridisation of a matched target binding region in contrast to a no-template control.

Figure 19 shows that random coiling of a primer of the invention is sufficient to bring the fluorophore and quencher together.

Figure 20 shows the bimolecular embodiment of the invention, different amounts of quencher oligonucleotide were added, (a) none, (b) 0.5μM, (c) 2μM and (d) 20μM.

Figure 21 shows (a) the proportion of free floating quencher to the Scorpions primer ie. 40X, 4X, 1X and 0X respectively, and (b) the effect of no quencher.

Examples

Materials

Primers/Scorpions primers:

B2098- BRCA Scorpions: FAM-CGCACGATGTAGCACATCAGAAGCGTGCG-MR-HEG-TTGGAGATTTTGTCACTTCCACTCTCAAA

Underlined regions are the hairpin forming parts, FAM is the fluorescein dye, MR is a non-fluorogenic fluorophore attached to a uracil, HEG is the replication blocking hexethylene glycol monomer. The probe matches the "C-variant" of the BRCA2 polymorphism and mismatches the "A-variant".

R186-98: untailed equivalent of B2098: TTGGAGATTTTGTCACTTCCACTCTCAAA

R187-98: opposing primer to the R186-98 and the equivalent Scorpions.

Z3702: the probe segment of the Scorpions B2098:

FAM-CGCACGATGTAGCACATCAGAAGCGTGCG-MR

Template DNA: previously genotyped DNA prepared by proteinase K and phenol/chloroform extraction was used at 50ng per 50μl reaction. Genotypes were typically one homozygous A/A, one homozygous C/C and one heterozygote (A/C).

Buffer (1x): 10 mM Tris-HCl (pH 8.3), 1.2 mM or 3.5 mM $MgCl_2$, 50 mM KCl, dNTPs (each at 100 μM), gelatin at 0.01% (w/v).

Enzyme: AmpliTaq Gold (Perkin-Elmer/ABI) was included in the reaction mix at 2units/50μl reaction.